AMENDMENTS TO THE SPECIFICATION

Sequence Listing:

Please amend the specification as follows:

On page 11, line 16; please delete - CGYGPKKKRKVGGC - , and insert -- SEQ ID 1 -- .

On page 34, starting on line 25:

please delete - H-CGYGPKKKRKVGG-OH - , and insert -- SEQ ID 2 -- ;

please delete - H-CKKKS-SSDDEATADSQHSTPPKKKRKVEDPKDFPSELLS-OH - ,

and insert -- SEQ ID 3 -- ;

please delete - H-CKKKWDD-EATADSQHSTPPKKKRKVEDPKDFPSELLS-OH - ,

and insert -- SEQ ID 4 -- ;

please delete - CYNDFGNYNNQSSNFGPMKQGNFGGRSSGPY -,

and insert -- SEQ ID 5 -- ;

please delete - H-CKRGPKRPRP-OH - , and insert -- SEQ ID 6 -- ;

please delete - H-CKKAVKRPAATKKAGQ-AKKKKL-OH - ,

and insert -- SEQ ID 7 -- ; and,

please delete - H-CKKKGPAAKRVKLD-OH - , and insert -- SEQ ID 8 -- .

On page 39, line 7 ; please delete - H_2N-KLLKLLLKLWLKLLKLLLKLL-CO_2 - ,

and insert -- SEQ ID 9 -- .

On page 72, line 9; after - H_2N-EEEEEEEE-$NHCH_2CH_2NH_2$ (- ,

please insert -- SEQ ID 10; -- .

On page 93, line 1; after - $AcKL_3$ - , please insert -- ; SEQ ID 9 -- .

On page 94, line 27; after - **$pAcKL_3$** - , please insert -- **; SEQ ID 9** -- .

The specification has been amended to include a sequence listing for the sequences found in the specification. Replacement sheets indicating the changes mad have been included. A sequence listing paper copy has been submitted with this Amendment as additional sheets to the specification. A computer readable form has also been submitted and it is the same as the paper copy that has been added. The sheets do not include new matter.

Respectfully submitted,

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associated with the template) are positioned along template's backbone, thereby promoting their polymerization. Weak electrostatic association of the nascent polymer and the template becomes stronger with chain growth of the polymer. Trubetskoy et al used two types of polymerization reactions to achieve DNA condensation: step polymerization and chain polymerization (VS Trubetskoy, VG Budker, LJ Hanson, PM Slattum, JA Wolff, LE Hagstrom. Nucleic Acids Res. 26:4178-4185, 1998) U.S. 08/778,657, U.S. 09/000,692, U.S. 97/24089, U.S. 09/070299, and U.S. 09/464,871. Bis(2-aminoethyl)-1,3-propanediamine (AEPD), a tetramine with 2.5 positive charges per molecule at pH 8 was polymerized in the presence of plasmid DNA using cleavable disulfide amino-reactive cross-linkers dithiobis (succinimidyl propionate) and dimethyl-3,3'-dithiobispropionimidate. Both reactions yielded DNA/polymer complexes with significant retardation in agarose electrophoresis gels demonstrating significant binding and DNA condensation. Treatment of the polymerized complexes with 100 mM dithiothreitol (DTT) resulted in the pDNA returning to its normal supercoiled position following electrophoresis proving thus cleavage the backbone of the. The template dependent polymerization process was also tested using a 14 mer peptide encoding the nuclear localizing signal (NLS) of SV40 T antigen (~~CGYGPKKKRKVGGC~~ SEQ ID 1) as a cationic "macromonomer". Other studies included pegylated comonomer (PEG-AEPD) into the reaction mixture and resulted in "worm"-like structures (as judged by transmission electron microscopy) that have previously been observed with DNA complexes formed from block co-polymers of polylysine and PEG (MA Wolfert, EH Schacht, V Toncheva, K Ulbrich, O Nazarova, LW Seymour. Human Gene Ther. 7:2123-2133, 1996). Blessing et al used bisthiol derivative of spermine and reaction of thiol-disulfide exchange to promote chain growth. The presence of DNA accelerated the polymerization reaction as measured the rate of disappearance of free thiols in the reaction mixture (T Blessing, JS Remy, JP Behr. J. Am. Chem. Soc. 120:8519-8520, 1998).

"Caging" of polycation-condensed DNA particles.

The stability of DNA nanoassemblies based on DNA condensation is generally low in vivo because they easily engage in polyion exchange reactions with strong polyanions. The process of exchange consists of two stages: 1) rapid formation of a triple DNA-polycation-polyanion complex, 2) slow substitution of one same-charge polyion with another. At equilibrium conditions, the whole process eventually results in formation of a new binary complex and an excess of a third polyion. The presence of low molecular weight salt can greatly accelerate such exchange reactions, which often result in complete disassembly of

An interpolyelectrolyte complex is a noncovalent interaction between polyelectrolytes of opposite charge.

Charge, Polarity, and Sign

The charge, polarity, or sign of a compound refers to whether or not a compound has lost one or more electrons (positive charge, polarity, or sign) or gained one or more electrons (negative charge, polarity, or sign).

Cell Targeting Signals

Cell targeting signal (or abbreviated as the Signal) is defined in this specification as a molecule that modifies a biologically active compounds such as drug or nucleic acid and can direct it to a cell location (such as tissue) or location in a cell (such as the nucleus) either in culture or in a whole organism. By modifying the cellular or tissue location of the foreign gene, the function of the biologically active compound can be enhanced.

The cell targeting signal can be a protein, peptide, lipid, steroid, sugar, carbohydrate, (non-expressing) polynucleic acid or synthetic compound. The cell targeting signal enhances cellular binding to receptors, cytoplasmic transport to the nucleus and nuclear entry or release from endosomes or other intracellular vesicles.

Nuclear localizing signals enhance the targeting of the pharmaceutical into proximity of the nucleus and/or its entry into the nucleus. Such nuclear transport signals can be a protein or a peptide such as the SV40 large T ag NLS or the nucleoplasmin NLS. These nuclear localizing signals interact with a variety of nuclear transport factors such as the NLS receptor (karyopherin alpha) which then interacts with karyopherin beta. The nuclear transport proteins themselves could also function as NLS's since they are targeted to the nuclear pore and nucleus. For example, karyopherin beta itself could target the DNA to the nuclear pore complex. Several peptides have been derived from the SV40 T antigen. These include a short NLS (~~H-CGYGPKKKRKVGG-OH~~ SEQ ID 2) or long NLS's (~~H-CKKKS-SSDDEATADSQHSTPPKKKRKVEDPKDFPSELLS-OH~~ SEQ ID 3 and ~~H-CKKKWDD-EATADSQHSTPPKKKRKVEDPKDFPSELLS-OH~~ SEQ ID 4). Other NLS peptides have been derived from M9 protein (~~CYNDFGNYNNQSSNFGPMKQGNFGGRSSGPY~~ SEQ ID 5), E1A (~~H-CKRGPKRPRP-OH~~ SEQ ID 6), nucleoplasmin (~~H-CKKAVKRPAATKKAGQ-AKKKKL-OH~~ SEQ ID 7),and c-myc (~~H-CKKKGPAAKRVKLD-OH~~ SEQ ID 8).

Signals that enhance release from intracellular compartments (releasing signals) can cause DNA release from intracellular compartments such as endosomes (early and late), lysosomes, phagosomes, vesicle, endoplasmic reticulum, golgi apparatus, trans golgi network (TGN), and sarcoplasmic reticulum. Release includes movement out of an intracellular

lactis), androctonin (scorpion), cardiotoxin I (cobra), caerin (frog litoria splendida), dermaseptin (frog). Viral peptides have also been shown to have membrane activity, examples include hemagglutinin subunit HA-2 (influenza virus), E1 (Semliki forest virus), F1 (Sendai and measles viruses), gp41 (HIV), gp32 (SIV), and vp1 (Rhino, polio, and coxsackie viruses). In addition synthetic peptides have also been shown to have membrane activity. Synthetic peptides that are rich in leucines and lysines (KL or KL_n motif) have been shown to have membrane activity. In particular, the peptide ~~H_2N-KLLKLLLKLWLKLLKLLLKLL-CO_2~~ SEQ ID 9, termed KL_3, is membrane active.

Compounds or Chemical Groups (Moieties) that Inhibit or Block the Membrane Activity of Another Compound or Chemical Moiety

An interaction with a membrane active agent by modification or complexation (including covalent, ionic, hydrogen bonding, coordination, and van der Waals bonds) with another compound that causes a reduction, or cessation of the said agents membrane activity. Examples include the covalent modification of a membrane-active peptide by the covalent attachment of an inhibitory chemical group (moiety) to the membrane active peptide. Another example includes the interpolyelectrolyte complexation of a membrane active polyanion and inhibitory polycation.

Polymers

A polymer is a molecule built up by repetitive bonding together of smaller units called monomers. In this application the term polymer includes both oligomers which have two to about 80 monomers and polymers having more than 80 monomers. The polymer can be linear, branched network, star, comb, or ladder types of polymer. The polymer can be a homopolymer in which a single monomer is used or can be copolymer in which two or more monomers are used. Types of copolymers include alternating, random, block and graft.

The main chain of a polymer is composed of the atoms whose bonds are required for propagation of polymer length. For example in poly-L-lysine, the carbonyl carbon, α-carbon, and α-amine groups are required for the length of the polymer and are therefore main chain atoms. The side chain of a polymer is composed of the atoms whose bonds are not required for propagation of polymer length. For example in poly-L-lysine, the β, γ, δ, and ε-carbons, and ε-nitrogen are not required for the propagation of the polymer and are therefore side chain atoms.

To those skilled in the art of polymerization, there are several categories of polymerization processes that can be utilized in the described process. The polymerization can be chain or step. This classification description is more often used that the previous

1,5-Hexafluoro-2,4-Pentanone - 1,4-Bis(3-aminopropyl)piperazine Copolymer (1:1) (MC339)

1,5-Hexafluoro-2,4-Pentanone - Tetraethylenepentamine Copolymer (1:1) (MC346).

Example V) Synthesis of Poly-L-Glutamic acid (octamer) - Glutaric Dialdehyde Copolymer (MC151).

H_2N-EEEEEEEE-$NHCH_2CH_2NH_2$ (SEQ ID 10; 5.5 mg, 0.0057 mmol, Genosis) was taken up in 0.4 mL H_2O. Glutaric dialdehyde (0.52 µL, 0.0057 mmol, Aldrich Chemical Company) was added and the mixture was stirred at room temperature. After 10 min the solution was heated to 70 °C. After 15 hrs, the solution was cooled to room temperature and dialyzed against H_2O (2x2L, 3500 MWCO). Lyophilization afforded 4.3 mg (73%) poly-glutamic acid (octamer) - glutaric dialdehyde copolymer.

Example W) Synthesis of Di-(2-methyl-4-aminomethyl-1,3-dioxolane)-1,4-benzene - Glutaric Dialdehyde Copolymer (MC352).

To a solution of di-(2-methyl-4-aminomethyl-1,3-dioxolane)-1,4-benzene (23 mg, 75 µmol) in dimethylformamide (200 µL) was added glutaric dialdehyde (7.5 mg, 75 µmol, Aldrich Chemical Company). The reaction mixture was heated at 80°C for 6 hrs under nitrogen. The solution was cooled to room temperature and used without further purification.

Example X) Synthesis of Di-(2-methy-4-hydroxymethyl(glycine ester)-1,3-dioxolane)-1,4,benzene - Glutaric Dialdehyde Copolymer (MC357).

To a solution of di-(2-methy-4-hydroxymethyl(glycine ester)-1,3-dioxolane)-1,4,benzene (35 mg, 82 µmol) in dimethylformamide (250 µL) was added glutaric dialdehyde (8.2 mg, 82 µmol, Aldrich Chemical Company). The reaction mixture was heated at 80°C for 12 hrs. The solution was cooled to room temperature and used without further purification.

KLLKLLLKLWLKLLKLLLKLL-CO_2 ($AcKL_3$; SEQ ID 9) was synthesized according to published procedure (O'Brien-Simpson, N.M., Ede, N.J., Brown, L.E., Swan, J., Jackson, D.C *J. Am. Chem. Soc.* **1997**, 119, 1183).

B) Coupling KL_3 to poly(allylamine).

To a solution of poly(allylamine) (2mg) in water (0.2 mL) was added KL3 (0.2 mg, 2.5 eq) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (1mg, 150 eq). The reaction was allowed to react for 16 h and then the mixture was placed into dialysis tubing and dialyzed against 3x1 L for 48 h. The solution was then concentrated by lyophilization to 0.2 mL.

C) Synthesis of L-cystine - 1,4-bis(3-aminopropyl)piperazine copolymer

To a solution of N,N'-Bis(t-BOC)-L-cystine (85 mg, 0.15 mmol) in ethyl acetate (20 mL) was added N,N'-dicyclohexylcarbodiimide (108 mg, 0.5 mmol) and N-hydroxysuccinimide (60 mg, 0.5 mmol). After 2 hr, the solution was filtered through a cotton plug and 1,4-bis(3-aminopropyl)piperazine (54 mL, 0.25 mmol) was added. The reaction was allowed to stir at room temperature for 16 h. The ethyl acetate was then removed by rotary evaporation and the resulting solid was dissolved in trifluoroacetic acid (9.5 mL), water (0.5 mL) and triisopropylsilane (0.5 mL). After 2 h, the trifluoroacetic acid was removed by rotary evaporation and the aqueous solution was dialyzed in a 15,000 MW cutoff tubing against water (2 X 2 l) for 24 h. The solution was then removed from dialysis tubing, filtered through 5 μM nylon syringe filter and then dried by lyophilization to yield 30 mg of polymer.

D) Synthesis of 5,5'-Dithiobis(2-nitrobenzoic acid) - 1,4-Bis(3-aminopropyl)piperazine Copolymer

1,4-Bis(3-aminopropyl)piperazine (10 mL, 0.050 mmol, Aldrich Chemical Company) was taken up in 1.0 mL methanol and HCl (2 mL, 1 M in Et2O, Aldrich Chemical Company) was added. Et2O was added and the resulting HCl salt was collected by filtration. The salt was taken up in 1 mL DMF and 5,5'-dithiobis[succinimidyl(2-nitrobenzoate)] (30 mg, 0.050 mmol) was added. The resulting solution was heated to 80 C and diisopropylethylamine (35 mL, 0.20 mmol, Aldrich Chemical Company) was added by drops. After 16 hr, the solution was cooled, diluted with 3 mL H2O, and dialyzed in 12,000 - 14,000 MW cutoff tubing

against water (2 X 2 L) for 24 h. The solution was then removed from dialysis tubing and dried by lyophilization to yield 23 mg (82%) of 5,5'-dithiobis(2- nitrobenzoic acid) - 1,4-bis(3-aminopropyl)piperazine copolymer.

E) Synthesis of polypropylacrylic acid

To a solution of diethylpropylmalonate (2 g, 10 mmol) in 50 mL ethanol was added potassium hydroxide (0.55 g, 1 eq) and the mixture was stirred at room temperature for 16 hours. The ethanol was then removed by rotary evaporation. The reaction mixture was partitioned between 50 mL ethyl acetate and 50 mL of water. The aqueous solution was isolated, and acidified with hydrochloric acid. The solution was again partitioned between ethyl acetate and water. The ethyl acetate layer was isolated, dried with sodium sulfate, and concentrated to yield a clear oil. To this oil was added 20 mL of pyridine, paraformaldehyde (0.3 g, 10 mmol), and 1 mL piperidine. The mixture was refluxed at 130 °C until the evolution of gas was observed, ca. 2 hours. The ester product was then dissolved into 100 mL ethyl ether, which was washed with 100 mL 1M hydrochloric acid, 100 mL water, and 100 mL saturated sodium bicarbonate. The ether layer was isolated, dried with magnesium sulfate, and concentrated by rotary evaporation to yield a yellow oil. The ester was then hydrolyzed by dissolving in 50 mL ethanol with addition of potassium hydroxide (0.55 gm, 10 mmol). After 16 hours, the reaction mixture was acidified by the addition of hydrochloric acid. The propylacrylic acid was purified by vacuum distillation (0.9 g, 80% yield), boiling point of product is 60 °C at 1 torr.

The propylacrylic acid was polymerized by addition of 1 mole percent of azobisisobutyonitrile and heating to 60 °C for 16 hours. The polypropylacrylic acid was isolated by precipitation with ethyl ether.

F) Synthesis of poly N-terminal acryloyl 6-aminohexanoyl-KLLKLLLKLWLKLLKLLLKLL-CO_2 ($pAcKL_3$; SEQ ID 9).

A solution of AcKL3 (20 mg, 7.7 μmol) in 0.5 mL of 6M guanidinium hydrochloride, 2 mM EDTA, and 0.5 M Tris pH 8.3 was degassed by placing under a 2 torr vacuum for 5 minutes. Polymerization of the acrylamide was initiated by the addition of ammonium persulfate (35 μg, 0.02 eq.) and N,N,N,N-tetramethylethylenediamine (1 μL). The polymerization was allowed to proceed overnight. The solution was then placed into dialysis tubing (12,000 molecular weight cutoff) and dialyzed against 3X2 L over 48 hours. The amount of